

New concepts in antimicrobial susceptibility testing: the mutant prevention concentration and mutant selection window approach

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Abstract

Current measurements of antimicrobial susceptibility or resistance utilize a standardized bacterial inoculum (10^5 cfu/mL) exposed to varying drug concentrations in a test tube. Following incubation under ideal conditions, the lowest drug concentration inhibiting growth is the minimum inhibitory concentration (MIC). When the MIC exceeds the amount of drug that can be safely achieved in the body, we call these microorganisms resistant; established breakpoints for various 'bug-drug' combinations are used to categorize microorganisms as susceptible, intermediate or resistant. MIC testing has been used for decades to guide antimicrobial therapy and remains an important measurement for infectious diseases. More recently, the mutant prevention concentration (MPC) has been described as a novel measurement of *in vitro* susceptibility or resistance and is based on the testing of larger bacterial inocula, i.e. $\geq 10^9$ cfu/mL – such as those associated with some infections in humans and animals. MPC defines the lowest drug concentration required to block the growth of the least susceptible cell present in high density bacterial populations. MPC testing applies to microorganisms considered susceptible to the drug by MIC testing. The mutant selection window (MSW) defines the 'danger zone' for therapeutic drug concentrations. Minimizing the length of time the drug concentration remains in the MSW may reduce the likelihood for resistance selection during therapy. The MSW is bordered by the MIC and MPC values and the drug concentration range between the measured MIC and MPC values defines the MSW. MPC values, when considered with drug pharmacology,

may allow prediction on the probability of resistance selection when bacteria are exposed to antimicrobial agents during therapy for infectious diseases. In today's environment, resistance prevention should be a goal of antimicrobial therapy.

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Introduction

Alexander Fleming – one of our forefathers of antimicrobial agents – had tremendous foresight about the use of these drugs. Since the initial introduction into clinical practice of antimicrobial agents, antimicrobial resistance has been a concern – a concern identified by Fleming himself. Fleming commented in 1945, '...But I would like to sound a note of warning...it is not difficult to make microbes resistant to penicillin in the laboratory by exposing them to concentrations not sufficient to euthanize them and the same thing has occasionally happened in the body.' Undoubtedly, Fleming was warning against exposing bacteria to insufficient concentrations of drug and that doing so would ultimately encourage resistance selection.

Today, there is little doubt that we have a global pandemic of antimicrobial-resistant microorganisms: in human infectious diseases, drug resistance concerns are seen with *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Acinetobacter* species and enteric Gram-negative bacilli expressing extended spectrum beta-lactamase (*Escherichia coli*, *Klebsiella* spp. and others).¹ Some of these same microorganisms are also a concern in veterinary medicine, as is *Staphylococcus pseudintermedius* (previously *intermedius*). While regional variations in resistance rates exist for various bacteria/drug combinations, the overall trend has impacted on individual cases, society, the economics of managing infections, and our approach to the empirical use of antimicrobial compounds for both inpatient and outpatient management.

In veterinary medicine, as in human medicine, antimicrobial agents are approved based on the demonstration of noninferiority of a new drug when compared to a standard antibiotic agent already approved for a specific indication. Clinical trials may fail to take into account various microbiological or pharmacological parameters that could be used to determine optimal versus suboptimal dosing and the potential for resistance selection or prevention. Such parameters may not necessarily affect clinical outcome, but may have a huge impact on the selection of

drug resistant pathogens. Clearly, our measurement of antimicrobial susceptibility and the resultant susceptibility or resistance result influences (or should influence) optimization of therapy.

Correlating *in vitro* measurements of antimicrobial susceptibility and the clinical impact

Johnson commented on the predictive value of *in vitro* clinical methods used to evaluate antimicrobial efficacy.² In hosts with normal immune defences, a microorganism susceptible to an antimicrobial agent, as indicated by a low minimum inhibitory concentration (MIC), has an excellent predictive value for a favourable clinical outcome. Antimicrobial resistance as indicated by a high MIC is usually predictive of an unfavourable outcome. Higher MICs usually indicate a greater likelihood of clinical failure. Similar points were made by Johnson regarding hosts with endocarditis, meningitis or deficient immune defences. This, therefore, suggests that the *in vitro* measurement of antimicrobial susceptibility has utility in clinical practice. Unfortunately, MIC testing utilizes a bacterial inoculum that may not be representative of bacterial burdens present during infection (urinary tract, respiratory tract, or central nervous system).³

Minimum inhibitory concentration testing

Determination of *in vitro* susceptibility of a pathogen to an antimicrobial agent can be performed by disk diffusion or by the measurement of the MIC, the lowest drug concentration inhibiting or blocking the growth of 10^5 colony forming units/mL (cfu/mL) of the bacterium. Susceptibility testing is controlled for incubation in or on appropriate media, atmosphere, temperature and duration of incubation. Methods for MIC testing include broth microdilution, agar dilution or the E-test. For broth microdilution testing, drug is added to medium in a 96 well microtitre tray and serially diluted to the desired drug concentration range to be tested. Following addition of microorganism, the assay is incubated for 18–24 h. The lowest drug concentration preventing visible growth is recorded as the MIC. For agar dilution testing, agar plates incorporating antimicrobial drug at pre-determined drug concentrations directly into the medium are inoculated with a known concentration of microorganisms to the surface of the agar plate. Following incubation, the lowest drug concentration inhibiting growth is the MIC. For E-test, the appropriate inoculum of microorganism is inoculated over the entire surface of an agar plate and an E-test strip containing gradations of drug concentrations is added to the surface of the plate and incubated. Following incubation, the point on the E-test strip that intersects the line of bacterial inhibition is recorded as the MIC. Antimicrobial susceptibility or resistance is then determined by comparing the measured MIC value to previously established breakpoints that take into account: (i) the drug's *in vitro* activity; (ii) achievable and sustainable drug concentrations within the host; (iii) distribution and elimination data; and (iv) drug toxicity. For an MIC recorded at or below the susceptibility breakpoint, the microorganism is considered susceptible.⁴ For MICs

recorded above the susceptibility breakpoint, the microorganism is classified as nonsusceptible or resistant. Readers should refer to the relevant Clinical and Laboratory Standard Institute (CLSI) recommended documents (i.e. CLSI document M31-A3) for breakpoints used in veterinary medicine.

In vitro susceptibility testing based on utilization of standardized bacterial inocula (10^5 cfu/mL) has been the foundation of susceptibility testing for decades. Most agree that this form of standardized susceptibility testing has been useful clinically and does serve as a guide for the management of cases with infectious diseases. The case group that benefits most from susceptibility testing remains debatable: outpatients with self limiting mild to moderate infections, versus inpatients with sepsis. While the correlation between an *in vitro* susceptibility result and clinical outcome is not 100% – other factors play a role in determining outcomes – the test is still useful. In some instances, cases treated with a seemingly appropriate antimicrobial (i.e. one to which the microorganism is susceptible *in vitro*) may still fail to respond clinically, while those treated with an inappropriate antibiotic (i.e. one to which the microorganism is resistant) may still show a favourable clinical response. *In vitro* measurements cannot account for the host's immune response which is necessary for successful recovery from infectious diseases. Antimicrobial agents remain an adjunct therapy to the host's natural defences.

New measurements of *in vitro* antimicrobial susceptibility

The mutant prevention concentration (MPC) was described by Dong *et al.*⁵ as a novel *in vitro* measurement of antimicrobial susceptibility, and takes into account the probability of mutant subpopulations being present in high density bacterial populations. Testing fluoroquinolones against *Staphylococcus aureus* and *Mycobacterium smegmatis* strains, these investigators determined that as the number of bacterial cells exposed to drug *in vitro* increased, two distinct regions in the concentration for inhibition of bacterial growth were recognized. The first region was approximated by the MIC drug concentration, at which and at higher drug concentrations, viable microorganisms could be isolated from drug containing agar plates. Upon molecular analysis, these organisms were found to have mutations conferring reduced susceptibility or resistance to the fluoroquinolone compound being investigated. The second region, the drug concentration that blocked the growth of these mutant cells, was termed the *mutant prevention concentration* (MPC). The MIC drug concentration is typically lower than the MPC drug concentration, suggesting that prevention of growth of mutant subpopulations from high density bacterial inocula requires higher drug concentrations. This was confirmed in a subsequent report on MPC measurements of fluoroquinolones against clinical isolates of *Streptococcus pneumoniae*.⁶

For drugs such as fluoroquinolones where resistance usually arises *de novo*, the MPC can be defined as the antimicrobial drug concentration that would require a

Table 1. Comparison of protocols for MPC testing of various microorganisms*

Microorganism	#Starter plates inoculated	Duration of incubation	Atmosphere	Subculture to liquid media [†]	Duration of incubation	Centrifugation required [‡]	Incubation duration to define endpoint
<i>S. pneumoniae</i>	6	18–24 h	5% CO ₂	Yes 500 mL THB	18–24 h	Yes	24–48 h
<i>S. aureus</i>	3	18–24 h	O ₂	Yes 100 mL MHB	18–24 h	No	24–48 h
<i>E. coli</i>	2–3	18–24 h	O ₂	Yes 100 mL MHB	18–24 h	No	24–48 h
<i>P. aeruginosa</i>	2–3	18–24 h	O ₂	Yes 100 mL MHB	18–24 h	No	24–48 h
<i>Klebsiella</i> spp.	2–3	18–24 h	O ₂	Yes 100 mL MHB	18–24 h	No	24–48 h
<i>Citrobacter</i> spp.	2–3	18–24 h	O ₂	Yes 100 mL MHB	18–24 h	No	24–48 h
<i>S. intermedius</i>	2–3	18–24 h	O ₂	Yes 100 mL MHB	18–24 h	No	24–48 h
<i>M. haemolytica</i>	4–5	18–24 h	O ₂	Yes 100 mL HTM	18–24 h	Yes	24–48 h
<i>P. multocida</i>	3–4	18–24 h	O ₂	Yes 100 mL MHB	18–24 h	No	24–48 h
<i>H. influenzae</i>	7–8	18–24 h	CO ₂	Yes 100 mL HTM	18–24 h	Yes	24–48 h

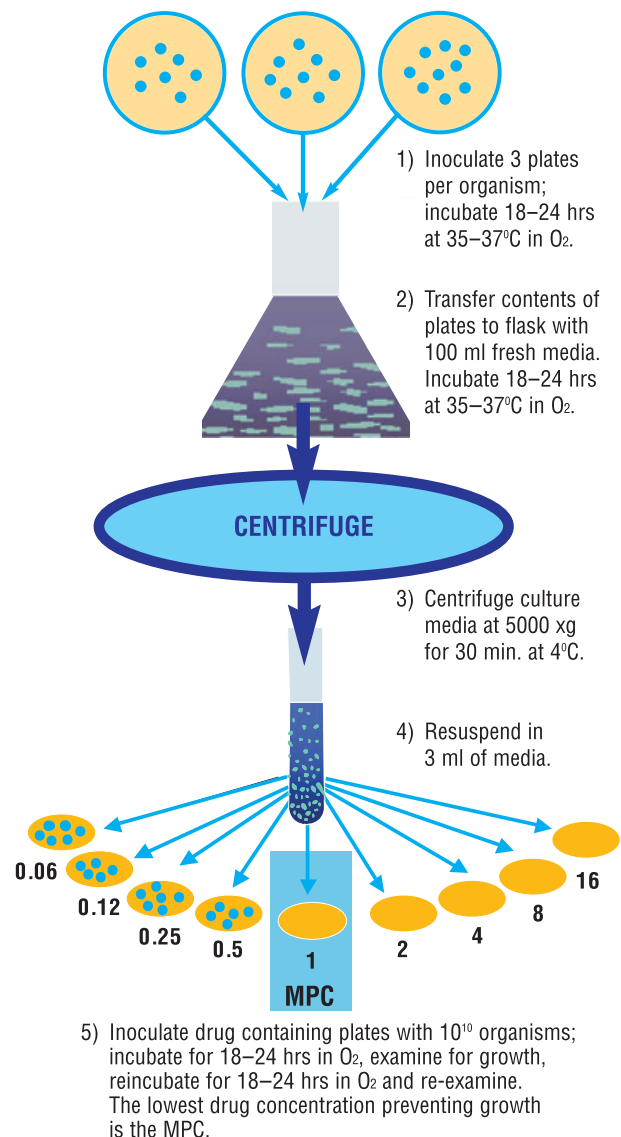
*Please see text for description.

[†]THB, Todd-Hewitt broth; MHB, Mueller–Hinton broth.[‡]5000 g for 10 min at 4 °C.

microorganism to possess two concurrent mutations at two different metabolic steps to grow in the presence of the drug. For fluoroquinolones, one can consider the MPC as the drug concentration required to block the growth of first step resistant mutants. MPC may also be defined as the MIC of the most resistant first step resistant cell present in the population. MPC measurements only apply to microorganisms deemed to be susceptible to an antimicrobial compound by current recommended susceptibility criteria and breakpoints.

Mutant prevention concentration testing is technically more demanding than MIC testing ($\geq 10^9$ cfus versus 10^5 cfu/mL respectively). MPC testing requires drug containing plates to be prepared, a centrifugation step may be required, and some organisms (i.e. *S. pneumoniae*) may be difficult to grow to a density of 10^{10} cfu/mL. Table 1 summarizes some comparative features of MPC testing of various microorganisms. Briefly, bacterial strains to be tested are subcultured to multiple agar plates (3–8) and incubated for 18–24 h under ambient conditions for the microorganism (i.e. O₂ versus 5% CO₂ at 35–37 °C). The next day, the complete contents of the inoculated agar plates are removed with a sterile swab and transferred to liquid broth (100–500 mL). The inoculated broth is then incubated for 18–24 h under ambient conditions. The next day, the broth culture either contains the necessary bacterial density or it must be centrifuged and the pellet resuspended in a lower volume of fresh broth media. Once the bacterial density is deemed to be correct, $\geq 10^9$ cfus are inoculated to drug containing agar plates and incubated under ambient conditions. Cultures are read at 24 and 48 h and the lowest drug concentration preventing growth is the MPC. A schematic diagram showing the method for MPC testing is shown in Figure 1.

Clearly, the current method of MPC testing is labour intensive and does not yet lend itself to easy implementation in clinical laboratories. Hesje and Blondeau⁷ compared a modified microbroth dilution method to the agar dilution method for determining MPCs.⁷ In this study, gatifloxacin and moxifloxacin were tested against a control strain of *Staphylococcus aureus* (American Type Culture Collection #29213) and against two clinical isolates. For this modified method, 10^1 – 10^9 cfu/mL test microorganisms were exposed to doubling drug

**Figure 1.** Schematic method of MPC testing.

concentrations in wells of microtitre plates. Following incubation under ambient conditions, the MIC was determined from the 10^5 cfu/mL inoculum and the MPC from inocula $\geq 10^7$ cfu/mL. By agar dilution

measurements, MPC values for all three strains were $\geq 4 \mu\text{g/mL}$ for these drugs. For inocula of 10^1 – 10^4 cfu/mL, MIC values ranged from 0.031 to $0.125 \mu\text{g/mL}$ and at 10^5 cfu/mL the MICs were 0.063 – $0.125 \mu\text{g/mL}$. At 10^7 – 10^9 cfu/mL, MPC values were $\geq 4 \mu\text{g/mL}$. For 10^1 – 10^4 cfu/mL, MICs to moxifloxacin ranged from 0.016 – $0.031 \mu\text{g/mL}$ and all strains had MICs of 0.031 at the 10^5 cfu/mL inoculum. For inocula of 10^7 – 10^9 cfu/mL, MPC values were $\geq 4 \mu\text{g/mL}$. Similar observations have been made with testing of gatifloxacin and moxifloxacin against a control strain and clinical isolates of *S. pneumoniae* (Blondeau, unpublished data). Further validation of this method is ongoing with microorganisms recovered from human and animal infections and tested against a broader range of antimicrobial agents. One potential additional step required with this new method relates to cellular debris present in wells at the higher bacterial densities. This may make visual interpretation difficult. In such situations, subculturing of the wells to a drug containing agar plate (same drug concentration as that in well of plate) may be necessary to confirm the endpoint.

The potential value of performing susceptibility measurements on higher bacterial populations

Firsch *et al.*⁸ estimated that in human with pneumococcal pneumonia, the total bacterial burden present during acute infection ranged from 10^{10} to 10^{12} microorganisms. This single observation alone suggests that many cases may be infected with greater numbers of bacterial organisms than the numbers used in current standardized MIC susceptibility testing. Subsequent to this, Feldman *et al.*⁹ reported bacterial counts in cerebral spinal fluid (CSF) ranging from 4.5×10^3 to 3×10^8 cfu/mL and suggested that persistence of a positive culture may be related to an initial high concentration of bacteria. Fagan *et al.*¹⁰ reported *Haemophilus influenzae* and *S. pneumoniae* bacterial counts of $\geq 10^7$ cfu/mL from protected brush specimens from cases with acute bacterial exacerbations of chronic bronchitis. Bingen *et al.*¹¹ reported CSF bacterial counts ranging from 2×10^7 to 4×10^9 cfu/mL; $\geq 10^7$ cfu/mL for *H. influenzae*, *N. meningitidis*, *S. pneumoniae*, *E. coli* K1 and *S. agalactiae*. Clearly, these reports suggest higher bacterial burdens during infection, prompting one to question if higher bacterial inocula should be used for susceptibility testing. It seems reasonable that higher bacterial burdens are also likely present in infected animals.

Since the description of the MPC concept by Dong *et al.*,⁵ numerous peer reviewed publications and abstracts have reported MIC and MPC values for various antimicrobial agents against gram-positive and Gram-negative microorganisms. A summary of MIC and MPC data is presented in Table 2. To date, the clinical significance of MPC measurements has not been fully elucidated; however, reports have been published showing the selection of drug resistant microorganisms (human pathogen *S. pneumoniae*) to the treatment drug during therapy.^{12,13}

Figures 2 and 3 show schematically the process of resistance selection when a high density bacterial popula-

tion is exposed to an antimicrobial agent. It is worth recalling that it only requires one spontaneous mutation to the exposed agent for the culture to become a $\geq 10^{12}$ population following overnight incubation. Figure 2 illustrates how rapidly one or two resistant strains can overcome the initially susceptible population, possibly leading to adverse clinical outcomes.

For cases with normal immunity (depicted in Figure 2 by letter B) both susceptible and resistant cells are cleared. In immunocompromised cases, those with prior infection, those with prior antimicrobial exposure or those cases that appear to be failing therapy for acute infection, it can be argued that continued proliferation of resistant microorganisms to the point where they breach the immune threshold may result in a bacterial population (depicted by letter A in Figure 2) with a predominance of resistant microorganisms. Alternatively, clearance and eradication may occur as part of the overall case response (depicted by C in Figure 2). In bovine respiratory disease, variables such as weather, shipment, co-mingling and other stressors may further compromise the animal and potentially pre-dispose them to prolonging the recovery from infections and thereby, increase the risk for resistance selection. As depicted in Figure 3, dosing based on MPC drug concentrations may reduce the overall bacterial numbers and also prevent the selective amplification of the resistance subpopulation if present as part of the total bacterial burden. MPC dosing may not reduce the likelihood that at risk cases may become infected with a new pathogen.

The 'danger zone' for the drug selective amplification of resistant subpopulations is postulated to occur in the mutant selection window (MSW) (Figure 4). We previously reported from *in vitro* experiments that drug concentrations exceeding the MPC drug concentration resulted in inhibiting susceptible and mutant microorganism growth.³ For drug concentrations falling below the MIC, neither mutant nor susceptible cells are inhibited. For drug concentrations falling within the MSW, susceptible cells are likely inhibited as the drug concentration is in excess of the MIC, however, mutant cells will not be inhibited as the drug concentration is below the MPC. Thus, therapeutic drug concentrations that lead to clinical cure may, in fact, be the same drug concentration that selectively amplifies the mutant fraction present in high density bacterial burdens. Dosing to achieve drug concentrations in excess of the MPC likely blocks susceptible and mutant cell growth.

In vitro euthanizing studies have been used to determine if an antimicrobial agent exhibits bacteriostatic versus bactericidal activity and as well, such studies were used to assess the extent and rate of euthanizing of antimicrobial agents. Historically, traditional euthanize studies were based on bacterial inocula of 10^5 cfu/mL and antibiotic drug concentrations that were multiples of the MIC (i.e. $1 \times$ MIC, $2 \times$ MIC, $4 \times$ MIC, $10 \times$ MIC, etc.). We previously argued that as bacterial burdens during infection exceed 10^5 cfu/mL then perhaps euthanize studies should be based on bacterial densities of 10^6 – 10^9 cfu/mL. Two such studies have been published to date detailing euthanize studies using higher bacterial inocula. In those reports with *S. pneumoniae* and fluor-

Table 2. Summary of MIC and MPC data. Modified from Hesje²⁶

Organism	Antimicrobial Agent	n	MIC ₉₀ *	MPC ₉₀ *	Reference	
<i>Streptococcus pneumoniae</i>	Moxifloxacin	99	ND	1 ⁺	6	
	Trovafloxacin	99	ND	2 ⁺	6	
	Gatifloxacin	100	ND	2 ⁺	6	
	Grepafloxacin	95	ND	4 ⁺	6	
	Levofloxacin	101	ND	4 ⁺	32	
	Ciprofloxacin	38	2	8	32	
	Ofloxacin	38	2	8	32	
	Azithromycin	177	0.125	4	33	
	Clarithromycin	206	0.063	1	33	
	Erythromycin	201	0.125	2	33	
	Pen susceptible	Moxifloxacin [§]	21	2	4	34
	Gatifloxacin [§]	21	4	8	34	
	Gemifloxacin [§]	21	0.25	2	34	
	Levofloxacin [§]	21	8	≥16	34	
	Azithromycin	49	0.5	≥8	35	
	Clarithromycin	49	≥1	≥4	35	
	Erythromycin	49	≥8	≥4	35	
	Pen intermediate	Garenoxacin	427	0.125	0.5	36
	Moxifloxacin	7	2	≥8	34	
	Gatifloxacin	7	4	≥16	34	
	Gemifloxacin	7	1	2	34	
Levofloxacin	7	8	≥32	34		
Azithromycin	10	>16	≥8	35		
Clarithromycin	10	≥1	≥4	35		
Erythromycin	10	≥8	≥4	35		
Pen resistant	Garenoxacin	80	0.125	0.5	36	
Gemifloxacin	8	0.063	0.5	37		
Levofloxacin	8	1	8	37		
Garenoxacin	17	0.063	0.5	36		
<i>Staphylococcus aureus</i>	MSSA	Ciprofloxacin	4	0.5	2	32
			1	0.125	2	38
		Gatifloxacin	4	0.063	0.063	32
			122	0.25	1	8
			1	0.016	0.125	38
		Ofloxacin	4	0.25	2	32
		Gemifloxacin	1	0.031	0.063	38
			122	0.063	0.5	8
		Levofloxacin	1	0.25	1	38
			122	0.25	1	8
		Moxifloxacin	1	0.015	0.25	38
			122	0.063	0.25	8
		Cefazolin	26	2	64	39
		Cloxacillin	26	0.25	2	39
		Vancomycin	26	1	4	39
	MRSA	Ciprofloxacin	1	0.125	1	38
		Gatifloxacin	1	0.063	0.125	38
			22	8	32	38
		Gemifloxacin	1	0.031	0.063	38
			22	8	256	28
		Levofloxacin	1	0.125	0.5	38
			22	>16	128	28
		Moxifloxacin	1	0.063	0.125	38
			22	4	16	28
		Cefazolin	24	16	512	39
		Cloxacillin	24	32	>512	39
		Vancomycin	24	1	8	39
	ATCC 6538	Pradofloxacin		0.03–0.06	0.5–0.6	40
		Danofloxacin		0.125–0.25	10–11	40
		Difloxacin		0.125	16–18	40
		Enrofloxacin		0.06–0.125	3–3.5	40
		Marbofloxacin		0.25–0.5	3–3.5	40
		Orbifloxacin		0.5	8–9	40
sarafloxacin			0.125–0.25	8–9	40	
Ciprofloxacin			0.25–0.5	6	40	
Moxifloxacin			0.03–0.06	0.8–1	40	
DSM 11823	Pradofloxacin		0.06	0.2–0.25	40	
	Enrofloxacin		0.125–0.25	1	40	

Table 2. (continued)

Organism	Antimicrobial Agent	n	MIC ₉₀ *	MPC ₉₀ *	Reference	
<i>Staphylococcus intermedius</i>						
ATCC 29663	Pradofloxacin		0.03	0.15	40	
	Enrofloxacin		0.06–0.125	2–2.5	40	
	Ciprofloxacin	1	0.03	0.25	A	
	Enrofloxacin	1	0.03	0.5	A	
	Amikacin		2	≥32	A	
	Ampicillin		0.25	≥128	A	
	Cefazolin		0.063	16	A	
	Doxycycline		2	≥64	A	
	Enrofloxacin		0.063	1	A	
	Erythromycin		0.5	≥8	A	
	Gentamicin		0.25	4	A	
	Marbofloxacin		0.5	1	A	
	Pradofloxacin		0.063	0.125	A	
	Ceftriaxone		1	8	A	
	cefodoxime		2	4	A	
	<i>Escherichia coli</i>					
		Ciprofloxacin	20	≤0.06	0.5	41
Levofloxacin		20	≤0.06	1	41	
Garenoxacin		20	≤0.06	1	41	
Moxifloxacin		23	0.031	1	42	
Tigecycline		26	0.063	1		
Amikacin			4	≥32	A	
Ampicillin			8	≥128	A	
Cefazolin			4	128	A	
Ceftriaxone			0.125	16	A	
Cefodoxime			0.125	16	A	
Doxycycline			1	≥64	A	
Enrofloxacin			0.016	0.25	A	
Gentamicin			1	≥8	A	
Marbofloxacin			0.016	0.5	A	
Pradofloxacin			0.016	0.125	A	
ATCC 8739		Pradofloxacin		0.015–0.03	0.2–0.25	40
		Danofloxacin		0.06	0.5–0.55	40
	Difloxacin		0.125–0.25	1.5–1.6	40	
	Enrofloxacin		0.03–0.06	0.3–0.35	40	
	Marbofloxacin		0.03	0.25–0.3	40	
	Orbifloxacin		0.125	1–1.25	40	
	Sarafloxacin		0.03–0.06	0.5–0.6	40	
	Ciprofloxacin		0.015–0.03	0.1–0.15	40	
	Moxifloxacin		0.06–0.125	0.5–0.6	40	
	Pradofloxacin		0.008–0.015	0.075–1	40	
	Enrofloxacin		0.015–0.03	0.15–0.175	40	
ATCC 2592	Marbofloxacin		0.015–0.03	0.2–0.25	40	
	Ciprofloxacin		0.008–0.015	0.1–0.15	40	
	Pradofloxacin		≤0.008	0.075–0.1	40	
	Enrofloxacin		≤0.008	0.125–0.15	40	
DMS 10650	Marbofloxacin		0.008–0.015	0.175–0.2	40	
	Ciprofloxacin		≤0.008	ND	40	
	Pradofloxacin		0.015–0.03	0.125–0.15	40	
Wild-type	Enrofloxacin		0.03–0.06	0.4–0.5	40	
	Marbofloxacin		0.03	0.5	40	
	Ciprofloxacin		0.015–0.03	0.3	40	
<i>Haemophilus influenzae</i>						
	Ciprofloxacin	31	0.016	0.5	43	
	Ofloxacin	31	0.031	0.5	43	
	Levofloxacin	31	0.016	0.125	43	
	Moxifloxacin	40	0.031	0.25	44	
	Gatifloxacin	31	0.031	0.125	43	
	Gemifloxacin	40	0.008	0.125	44	
	Azithromycin	40	2	32	44	
	Telithromycin	40	2	16	44	
	Clarithromycin	40	8	≥64	44	
	Cefuroxime	40	16	≥16	44	
	Ciprofloxacin	26	0.016	0.5	45	
	Ofloxacin	26	0.031	0.5	45	
	Moxifloxacin	26	0.031	0.5	45	
	Gatifloxacin	26	0.016	0.5	45	
	<i>Citrobacter freundii</i>					
	Ciprofloxacin	20	0.125	2	41	
	Levofloxacin	20	0.5	2	41	

Table 2. (continued)

Organism	Antimicrobial Agent	n	MIC ₉₀ *	MPC ₉₀ *	Reference	
<i>Enterobacter cloacae</i>	Garenoxacin	20	4	8	41	
	Ciprofloxacin	20	≤0.06	1	41	
	Levofloxacin	20	0.125	4	41	
<i>Klebsiella pneumoniae</i>	Garenoxacin	20	1	>8	41	
	Ciprofloxacin	20	≤0.06	1	41	
	Levofloxacin	20	1	2	41	
	Garenoxacin	20	0.25	4	41	
	Moxifloxacin	18	0.25	≥2	42	
	Ciprofloxacin	20	0.06	1	41	
<i>Klebsiella oxytoca</i>	Levofloxacin	20	1	2	41	
	Garenoxacin	20	0.25	4	41	
<i>Klebsiella spp.</i>	moxifloxacin	18	0.25	≥2	41	
	Moxifloxacin	6	0.063	0.5	42	
<i>Pseudomonas aeruginosa</i>	Tigecycline	24	0.5	8	46	
	Ciprofloxacin	20	1	4	41	
	Levofloxacin	20	4	16	41	
	Garenoxacin	20	4	≥32	41	
	Ofloxacin	22	8	16	43	
	Gatifloxacin	22	4	8	43	
	Ciprofloxacin	151		4	47	
	Levofloxacin	151		16	47	
	<i>Mannheimia haemolytica</i>	Enrofloxacin	139	0.125	0.5	29
		Florfenicol	135	2	4	29
Tilmicosin		143	8	≥32	29	
Tulathromycin		139	1	8	29	
<i>Salmonella typhimurium</i>	ciprofloxacin	1	0.03	0.5	48	
	enrofloxacin	1	0.03	8	48	

*Individual MIC or MPC values where only one organism was reported.

†Adjusted from two-fold overestimation in original publication.

‡Against organisms with elevated MICs to levofloxacin of ≥2 mg/L.

MIC, minimum inhibitory concentration; MPC, mutant prevention concentration; MRSA, methicillin Resistant *S. aureus*; MSSA, Methicillin-susceptible *S. aureus*; ND, not determined.

A, Blondeau unpublished.

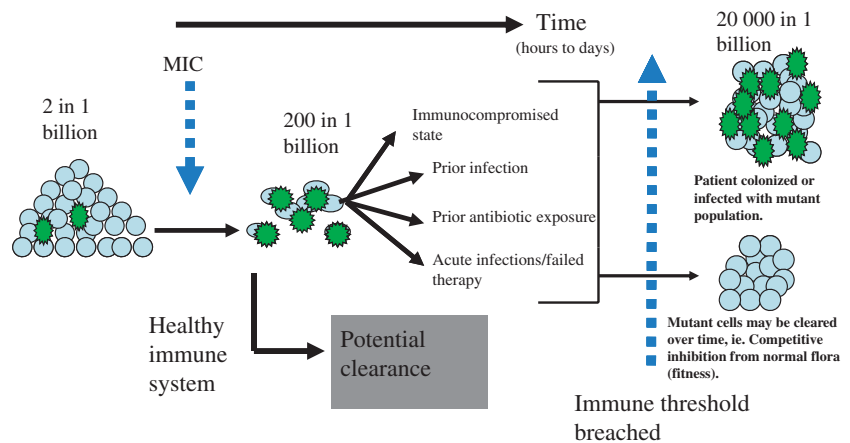
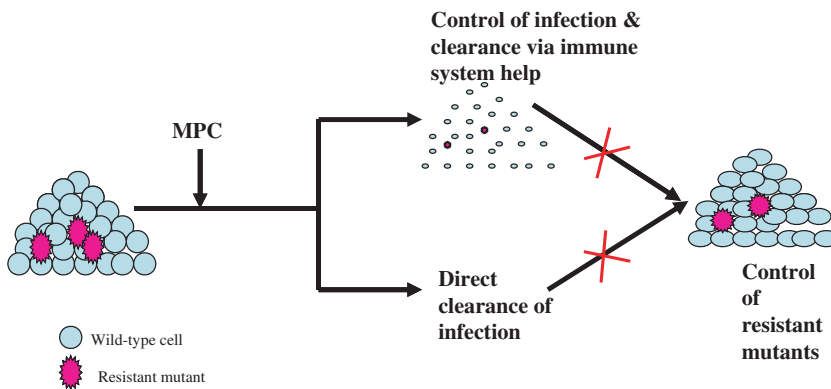


Figure 2. Schematic representation of resistance selection based on MIC drug concentrations.

quinolones, it was shown that euthanizing of 10^6 – 10^9 cfu/mL using the measured MIC drug concentration was slow and incomplete and in some instances, microorganism growth occurred in the presence of the drug concentration tested. Such observations are not completely surprising as the MIC is a measurement of inhibition of growth versus euthanizing, however, \log_{10} reductions are measurable at this drug concentration, indicating euthanizing occurs. When euthanizing of 10^6 – 10^9 cfu/mL was attempted with MPC drug concentrations, euthanizing was more rapid and complete suggesting MPC drug

concentrations were necessary to effect >99% reduction in high density bacterial populations.

Blondeau *et al.*¹⁴ compared the euthanizing of bovine isolates of *Mannheimia haemolytica* by enrofloxacin, florfenicol, tilmicosin and tulathromycin using the measured MIC and MPC drug values. In these experiments, microorganisms were grown to densities of 10^9 cfu/mL and then diluted to give densities ranging from 10^6 to 10^9 cfu/mL. Bacterial cultures were exposed to either the MIC or MPC drug concentrations, aliquots were sampled in triplicate at 0, 30 min, 1, 2, 3, 4, 12 and



During infection, both susceptible cells and first-step resistant mutants are present. Dosing at the level of the MPC would result in either direct clearance of infection via lytic drug action, or control of the infection facilitation clearance via the immune system.

Figure 3. Selective amplification of resistant mutants.

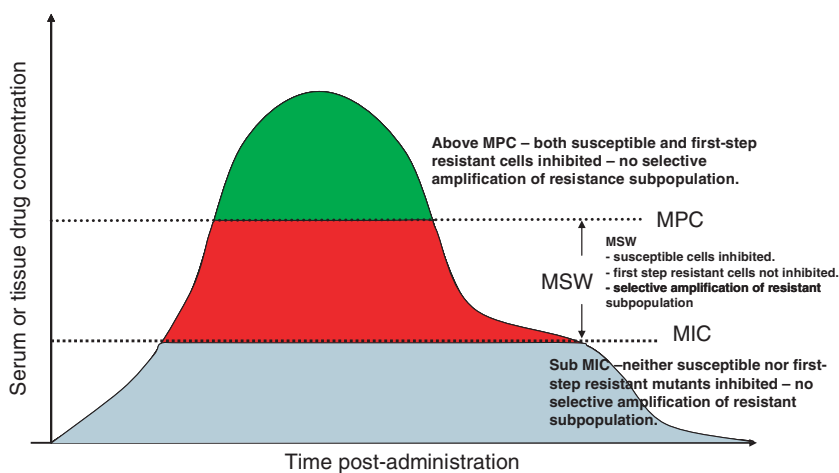


Figure 4. Mutant selection window (MSW). Reproduced with permission from reference 3.

24 h, plated, incubated under ambient conditions and the reductions in viable microorganisms recorded. In these experiments, the MIC values ($\mu\text{g}/\text{mL}$) for enrofloxacin, florfenicol, tilmicosin and tulathromycin were 0.16, 0.25–2, 0.5–4 and 0.2–2 respectively; MPC values ($\mu\text{g}/\text{mL}$) were 0.125–0.5, 2–8, 4–64 and 2–4 respectively. Exposure of 10^6 – 10^9 cfu/mL to the MIC drug concentrations gave growth to 2.4 \log_{10} reduction in viable cells by 4 h for florfenicol compared to growth to 0.13 \log_{10} reduction, growth to 0.57 \log_{10} reduction, growth to 0.35 \log_{10} reduction for enrofloxacin, tilmicosin and tulathromycin respectively. All drugs yielded a growth to 1.37 \log_{10} reduction of the 10^9 cfu/mL inocula by 24 h. Exposure of 10^6 – 10^9 cfu/mL of microorganism to MPC drug concentrations gave a growth to 3 \log_{10} reduction, growth to 0.49 \log_{10} reduction and growth to 0.1 \log_{10} reduction in viable cells by 30 min to 1 h for enrofloxacin, florfenicol, tilmicosin and tulathromycin respectively. A growth to 6 \log_{10} reduction was seen to the four drugs by 12–24 h with enrofloxacin showing the greatest reductions followed by florfenicol, tilmicosin and tulathromycin. It was concluded from this study, that euthanizing of the *M. haemolytica* strains was less efficient at the MIC drug

concentrations but was more complete and efficient at MPC drug concentrations as described above. Dosing to achieve MPC minimizes resistance selection and ensures more efficient and rapid euthanizing.

In a follow up study, the same authors examined the concentration dependent euthanizing of *M. haemolytica* isolates by enrofloxacin and in addition to conducting euthanize studies based on the MIC and MPC drug concentration values, drug concentration values representing the maximum serum and maximum tissue drug concentrations were also used in euthanize assays involving 10^6 – 10^9 cfu/mL.¹⁵ When *M. haemolytica* was exposed to enrofloxacin at the maximum serum drug concentration, a 1.7–2.4 \log_{10} reduction (96–99% euthanizing) was seen at 1 h. Similar values were also seen following exposure to the maximum tissue drug concentration. This study suggests that for concentration dependent antibiotics, dosing to achieve drug concentrations at or above the MPC drug concentration is necessary to effect a substantial reduction in viable microorganisms – especially high bacterial burdens such as those seen during infection.

Hansen *et al.*¹⁶ suggested that the period during which drug concentrations remain in excess of the MPC may be important for restricting mutant growth. In studies pub-

lished with *S. pneumoniae*, a > 99% reduction in viable cells occurred between 6 and 12 h of exposure to various fluoroquinolones when microorganisms were exposed to the MPC drug concentration in time euthanize experiments.^{17,18} Investigations with macrolide compounds showed similar results, i.e. that a minimum amount of time at or above the measured MPC value was necessary to effect substantial reduction in viable microorganisms.¹⁹ Euthanizing high density bacterial populations (10^6 – 10^9 cfu/mL) with MIC drug concentration is slow and incomplete. Such data might suggest that time above the MSW of at least 6 h may be important for ensuring substantial reductions of high density bacterial inocula as the euthanize experiments highlighted above were performed using bacterial inocula ranging from 10^6 – 10^9 cfu/mL – inocula consistent with the MPC approach.

In summary, MPC testing is a unique approach to *in vitro* susceptibility testing as it utilizes bacterial inocula which better reflect bacterial burdens present in a number of infections. Such testing might provide greater insight into the true dynamics of these high density bacterial populations when exposed to certain antimicrobial compounds.

Smith *et al.*²⁰ suggested that the MPC method of testing only applies to fluoroquinolone compounds. Subsequently, numerous studies have elucidated MPCs against a wide variety of antimicrobial compounds and bacterial pathogens. Molecular explanations of elevated MPC values remain unresolved for many 'bug-drug' combinations. A number of these observations are summarized in Table 2. The *resistance prevention concentration* (RPC) was coined as an all encompassing terminology to define the antimicrobial drug concentration that blocked the growth of the least susceptible microorganisms present in high density bacterial inocula and was independent of the mechanism of resistance of those mutant cells.³ In fact, MPC and RPC testing is synonymous and it remains important to remember that MPC defines the mutant prevention concentration and not the *mutation* prevention concentration. The measurement of MPC is to determine the drug concentration necessary to block the growth of the least susceptible cell in the population and is independent of the mechanism of resistance.

Not all mechanism of antimicrobial resistance arise *de novo*. In many instances, resistance may be the result of horizontal gene transfer by an acquired genetic element (plasmid, transposon) containing resistance-conferring genes. Such examples include beta-lactamase resistance on plasmids and tetracycline resistance on transposons. MPC measurements in these instances are unlikely to apply as microorganisms harbouring resistance conferring genes already demonstrate elevated MICs and are resistant by CLSI criteria. MPC testing is only relevant against bacterial strains susceptible to the drug by CLSI criteria. Once the organism is considered resistant by MIC testing, MPC measurements are not useful. While for some 'bug-drug' combinations the major mechanism of resistance is by the acquisition of a resistance gene, this does not exclude the potential for other mechanisms of resistance that are potentially preventable by MPC testing of susceptible strains.

Observations for fluoroquinolones in dermatology

For many antimicrobial agents, information regarding achievable or sustainable drug concentrations in the skin are somewhat elusive. For two fluoroquinolones – enrofloxacin and marbofloxacin – skin drug concentrations are available. For enrofloxacin, skin drug concentrations range from 1.7 to 1.9 µg/mL in dogs and cats and in inflamed skin in the dog, drug concentrations are 3.1 µg/mL after 3 days of therapy. As such, for *S. intermedicus*, an organism with an MIC of 0.063 µg/mL would yield a maximum tissue to MIC ratio between 26.9 and 30.1. As indicated in Table 2, the MPC for enrofloxacin against *S. intermedicus* was 0.5 µg/mL which would yield a tissue max/MPC ratio of 3.4 : 6.2.

For marbofloxacin, skin drug concentrations in dogs were 3.2 µg/mL and the tissue max/MIC ratio would be 6.4 (at an MIC of 0.5 µg/mL; Table 2). For an MPC of 1 µg/mL (Table 2), the tissue max/MPC ratio would be 3.2.

For both fluoroquinolones above, it is clear that higher or lower MIC and/or MPC values would influence the magnitude of the tissue max/MIC or MPC ratios. The exact clinical impact of these observations has not yet been studied. Regarding resistance prevention, higher ratios are likely to be more effective, however, this has not been evaluated clinically.

Minimum inhibitory concentration and mutant prevention concentration measurements need to be considered along with pharmacokinetic/pharmacodynamics data

The action of antimicrobial agents are clearly affected by pharmacokinetic (PK) and pharmacodynamics (PD) parameters, with PK defining the fate of the drug in the body (e.g. absorption, transformation, distribution and elimination) and PD defining the effect of the drug on the body and infecting organisms (including the drug's mechanism of action and efficacy).²¹

PK/PD principles have been used to characterize various compounds based on the mechanism by which they exert their antibacterial activity. Figure 5 shows the schematic representation of a drug curve. Three PK/PD relationships have been established and applied to various classes (and within classes to specific agents/species) of antimicrobial compounds. For *concentration dependent* antimicrobial compounds, the C_{max} to MIC ratio as well as the area under the curve (AUC) to MIC ratio have been shown to be important predictors of outcome following antimicrobial therapy. Antimicrobial compounds that are considered *time dependent* agents exert their antibacterial activity based on the time the drug concentration remains in excess of the MIC. A C_{max} to MIC ratio of >8–12 is felt to be important for positively impacting clinical outcome and reducing the likelihood for resistance selection. For agents characterized based on a AUC/MIC ratio, a ratio of >125 has been suggested as being necessary for Gram-negative microorganisms and 30–50 for gram-positive pathogens. The absolute values of these ratios

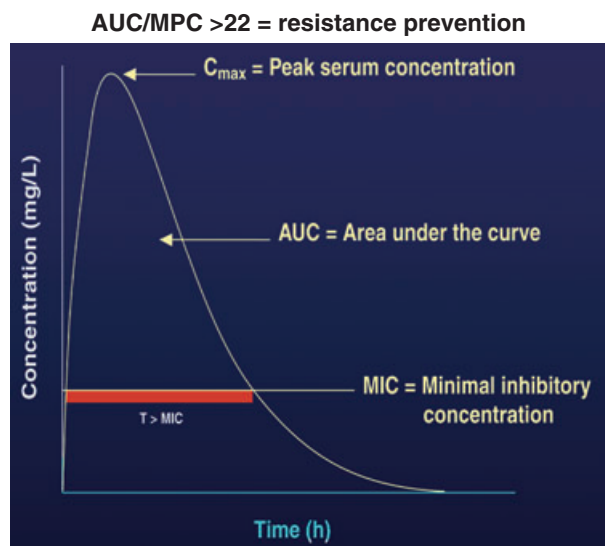


Figure 5. PK/PD Relationships: Surrogate markers.

Table 3. Antimicrobial agents and PK/PD characteristics²⁵

C_{max}/MIC	AUC/MIC	T>MIC
Streptomycin	Streptomycin	Benzylpenicillin
Gentamicin	Gentamicin	Amoxicillin
Tobramycin	Amikacin	Cloxacillin
Amikacin	Tobramycin	Carbenicillin
Danofloxacin	Danofloxacin	Cefalexin
Enrofloxacin	Enrofloxacin	Ceftiofur
Marbofloxacin	Marbofloxacin	Cephapirin
Difloxacin	Difloxacin	Florfenicol
Sarafloxacin	Sarafloxacin	Chloramphenicol
Metronidazole	Metronidazole	Erythromycin
	Colistin	Tilmicosin
	Oxytetracycline	Tulathromycin
	Chlortetracycline	Aivlosin
	Doxycycline	Clindamycin
	Azithromycin	Sulfadiazinesulfadoxime
	Clarithromycin	Trimethoprim
	Vancomycin	

C_{max}/MIC , maximum serum drug concentration to MIC ratio;
 AUC/MIC, area under the drug concentration curve to MIC ratio;
 T>MIC: time serum drug concentrations exceed the MIC over the dose.

have been debated and have yet to be completely resolved.^{22–24} Table 3 lists antimicrobial agents used in human and veterinary medicine and summarize the PK/PD parameters that characterize their mechanism of antimicrobial action.²⁵

It is clear that in patients being treated with antimicrobials, a number of possible scenarios may occur:²⁶

- 1 Clinical resolution with complete eradication of the pathogen from the infected site.
- 2 Clinical resolution with persistence of the microorganism in the host.
- 3 Clinical resolution with persistence of the pathogen that now is resistant to the treatment antimicrobial.
- 4 Clinical failure with microorganism proliferation.
- 5 Clinical failure with proliferation of an antimicrobial resistant pathogen.
- 6 Clinical failure due to infection with a secondary pathogen.

Optimal antimicrobial therapy would be that which results in a favourable clinical outcome where the infected microorganism has been eliminated and resistance selection prevented. Does it seem likely that a different AUC/MPC value would apply for resistance prevention against a gram-positive versus a Gram-negative organism? Unfortunately, such data is not readily available. Zinner *et al.*²⁷ used an *in vitro* pharmacodynamic model to test moxifloxacin against *S. pneumoniae* isolates and suggested that an $AUC_{24}/MIC >100$ may protect against selection of resistant *S. pneumoniae* mutants. Unfortunately, this value does not tell us what the AUC/MPC value might have been. Metzler *et al.*²⁸ determined MIC and MPC values for meticillin-susceptible strains of *Staphylococcus aureus* tested against gatifloxacin, gemifloxacin, levofloxacin and moxifloxacin. AUC_{0-24}/MPC_{90} ratios were calculated for total and free drug and were as follows respectively: 51.3/41, 16.8/6.7, 48/35.5 and 190/119.7. Unfortunately, it is uncertain as to what these values actually mean given that specific studies to investigate what the AUC/MPC values for *S. aureus* and fluoroquinolones need to be, has yet to be determined. Similarly, Blondeau *et al.*³ calculated AUC_{0-24}/MPC_{90} ratios for the same four fluoroquinolones against clinical isolates of *S. pneumoniae*. Those values respectively were 26.7, 18.4, 12 and 47.5: free drug values respectively would be 21.4, 7.4, 8.8 and 29.9. Once again, it remains unclear as to the significance of these values given that the appropriate studies have yet to be completed to determine what the AUC/MPC values need to be.

At least one study has compared MIC and MPC values for bovine isolates of *M. haemolytica* against enrofloxacin, florfenicol, tilmicosin and tulathromycin. In this report, MIC_{90} values ($\mu\text{g}/\text{mL}$) were 0.125, 0.5, 8 and 1 respectively; MPC_{90} values ($\mu\text{g}/\text{mL}$) were 0.5, 4, ≥ 32 and 8 respectively. Similar calculations have now been completed for enrofloxacin and *M. haemolytica*; AUC/MIC and AUC/MPC ratios were reported to be 160 and 80 respectively.²⁹ As florfenicol, tilmicosin and tulathromycin are classified as time dependent antimicrobials, $T>MIC_{90}$ and $T>MPC_{90}$ for these three agents were as follows respectively: 64 and ~ 3 h; >24 – <48 and 0 h; ≥ 172 and 0 h.

Olofsson *et al.*³⁰ investigated the selection of ciprofloxacin resistance in *Escherichia coli* in an *in vitro* kinetic model to determine the relationship between drug exposure and MPC. Two ciprofloxacin susceptible strains and one strain containing a first-step gyrase mutation were evaluated. The parameters investigated included $T>MPC$ ($T>MPC$), C_{max} and AUC/MPC. From their investigations, the authors concluded that neither of $T>MPC$ nor C_{max} proved to be single correlates for preventing resistance development in their experiments and against the strains tested. Against the two wild type susceptible strains, the authors found that an AUC/MPC ratio of ≥ 22 was a single pharmacodynamic index that predicted prevention of resistant mutant selection. The authors also concluded that further studies are warranted to verify the usefulness of this pharmacodynamic index for the design of dosing regimens. As yet, similar types of experiments have not been published related to gram-positive pathogens, therefore, it remains unclear if a dif-

ferent AUC/MPC value would be necessary for gram-positives versus Gram-negatives as has been argued for AUC/MIC values – particularly with *S. pneumoniae*. More recently, measurements of MPC have been completed with various veterinary pathogens and antimicrobial agents. A summary of these values are presented in Table 2.

As previously stated, optimal antimicrobial therapy would be that which results in a favourable clinical outcome, eradicates the infecting pathogen while minimizing likelihood for resistance selection during drug exposure. However, such a strategy may be prevented by adverse events observed at these higher, but microbiologically necessary drug concentrations. Additionally, higher dosages needed to prevent resistance development may cost more. Unfortunately, use of agents which fail to cure without resistance selection are likely to drive costs higher overall as clinicians have to use more expensive agents to overcome resistant pathogens. Treatment guidelines for a variety of infectious diseases in humans are beginning to address a new approach to prescribing antibiotics based on a century old concept; that of hitting hard and hitting fast, however, formalized guidelines have not materialized in veterinary medicine.³¹ Using the highest, safest antibiotic drug concentrations may provide excellent clinical outcomes with minimal side effects while preserving the drug class for future cases. Application of PK/PD to MPC concepts to avoid selecting mutant strains within bacterial populations can help improve both short term and long term outcomes.

In summary, MIC testing remains a useful guide for determining an organism's susceptibility to antimicrobial agents and has been the cornerstone of susceptibility testing for decades. Unfortunately, MIC testing may not fully take into account the true dynamics of higher density bacterial populations such as those associated with infection. MIC testing at an inoculum of 10^5 cfu/mL does not allow for the detection of resistant sub-populations that may arise in bacterial populations 10^6 – 10^9 CFUs or higher. As such, MPC testing may offer some value for guiding optimal antimicrobial therapy as it provides practical information on drug concentrations necessary to restrict mutant growth during infections where high bacterial burdens are likely present. Restricting mutant growth is desirable – especially given increasing antimicrobial resistance trends.

Measurements of MPC are often done using a single drug tested against one strain of bacteria. In some instances, single drug therapy may be insufficient and a combination of drugs may be warranted. Combination drug therapy was previously measured by us with clinical isolates of *Pseudomonas aeruginosa*. MPC values for either drug alone were outside of clinically achievable drug concentrations but combination MPC values were within achievable drug concentrations. This observation may have important implications for therapy against more difficult to treat pathogens or against organisms where multiple resistance mechanisms are common.

Sir Alexander Fleming wrote in 1946 '...the greatest possibility of evil in medication is the use of too small doses so that instead of clearing up infection the microbes are educated to resist penicillin and a host of

penicillin-fast organisms is bred out, which can be passed to other individuals and from them to others, until they reach someone who gets septicemia or pneumonia which penicillin cannot save.' Clearly, many unanswered questions exist regarding the MPC and MSW concepts. Further studies will be necessary to refine our understanding of the MSW and strategies to narrow or close the window so as to minimize the amount of time that drug concentrations remain in the 'danger zone'.

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Résumé Les mesures actuelles de la susceptibilité ou de la résistance antimicrobienne utilisent un inoculum bactérien standard (10^5 cfu/ml) soumis à des concentrations variables de médicament dans un tube à essai. Après incubation dans des conditions idéales, la concentration minimale de la molécule inhibant la croissance est appelée concentration minimale inhibitrice (MIC). Lorsque la MIC est supérieure à celle que la molécule peut atteindre dans l'organisme, on dit que ces micro-organismes sont résistants; des paliers ont été définis en fonction des conditions et permettent de distinguer les micro-organismes susceptibles, intermédiaires ou résistants. La mesure du MIC est utilisée depuis des décennies pour guider les thérapies antimicrobiennes et reste une mesure importante dans le cadre des maladies infectieuses. Récemment, la concentration de prévention de mutant (MPC) a été décrite comme une nouvelle mesure de susceptibilité ou de résistance *in vitro* et est basée sur une inoculation bactérienne plus large, i.e. $\geq 10^9$ cfu/ml, telles que celles associées à certaines infections humaines et animales. La MPC définit la plus basse concentration de médicament nécessaire pour arrêter la croissance cellulaire de la moins susceptible des populations bactériennes présente en grande densité. La MPC s'applique aux micro-organismes considérés comme susceptibles par la MIC. La fenêtre de sélection de mutants (MSW) définit la « zone de danger » pour les concentrations thérapeutiques. Réduire au minimum la durée pendant laquelle la concentration reste dans la MSW peut réduire la sélection de résistance au cours du traitement. La MSW est bordée par les valeurs de la MIC et de la MPC et l'échelle de concentrations de médicament comprises entre les valeurs de MIC et de MPC définissent la MSW. Les valeurs de la MPC, en considérant les données pharmacologiques, peuvent permettre de prédire la probabilité de sélectionner des résistances quand les bactéries sont exposées aux agents antimicrobiens au cours d'une thérapie anti-infectieuse. Dans notre environnement actuel, la prévention des résistances devrait être un des objectifs d'une thérapie antimicrobienne.

Resumen Las medidas actuales de susceptibilidad o resistencia antimicrobiana utilizan un inóculo bacteriano estándar (10^5 cfu/ml) expuesto a concentraciones variables de fármacos en un tubo de ensayo. Tras la incubación bajo condiciones ideales, la concentración mas baja inhibitoria de crecimiento es la concentración mínima inhibitoria (MIC). Cuando la MIC excede la cantidad de fármaco que se puede obtener en el cuerpo sin efectos adversos llamamos a los microorganismos resistentes; se utilizan puntos límite preestablecidos para varias condiciones "bacteria-fármaco" que categorizan a los microorganismos como susceptibles, intermedios o resistentes. Los ensayos de MIC se han utilizado durante décadas para guiar la terapia antimicrobiana y constituyen una medida importante para las enfermedades infecciosas. Más recientemente, la concentración preventiva de mutantes (MPC) se ha descrito como una nueva medida de la susceptibilidad o resistencia *in vitro* y se basa en el ensayo con mayores inóculos bacterianos, normalmente $>10^9$ cfu/ml –similares a las concentraciones asociadas con algunas infecciones en humanos y animales. La MPC define la concentración mínima de fármaco requerida para bloquear el crecimiento de la bacteria menos susceptible presente en poblaciones bacterianas de alta densidad. El ensayo de MPC se aplica a microorganismos considerados susceptibles al fármaco en el ensayo de MIC. La ventana de selección mutante (MSW) define la "zona de peligro" para la concentraciones terapéuticas de fármaco. Disminuyendo el tiempo que la concentración de fármaco permanece en la MSW podría reducir el riesgo de selección de resistencias durante el tratamiento. La MSW es intermedia entre los valores de MIC y MPC y el rango de concentración de fármaco entre el valor de MIC y MPC define la MSW. Los valores de MPC, cuando se consideran en la farmacología del compuesto, pueden permitir predicción de la probabilidad de selección de resistencia cuando las bacterias son expuestas a agentes antimicrobianos durante la terapia frente a enfermedades infecciosas. En el clima actual, la prevención de la resistencia debe ser un objetivo de la terapia antimicrobiana.

Zusammenfassung Zurzeit wird für die Messung von antimikrobieller Empfindlichkeit oder Resistenz ein standardisiertes bakterielles Inoculum verwendet (10^5 fcu/ml), das im Teströhrchen verschiedenen Wirkstoffkonzentrationen ausgesetzt wird. Nach der Inkubation unter idealen Bedingungen wird die niedrigste Wirkstoffdosis, die ein Wachstum verhindert, als minimale inhibitorische Konzentration bezeichnet (MIC). Wenn die MIC die Menge an Wirkstoff überschreitet, die sicher im Körper erreicht werden kann, nennen

wir diese Mikroorganismen resistent; festgelegte Messpunkte für verschiedene „bug-drug“ (Bakterien-Wirkstoff) Konditionen werden verwendet, um Mikroorganismen als empfänglich, intermediär oder resistent zu bezeichnen. Die MIC Untersuchung wird seit Jahrzehnten eingesetzt, um antimikrobielle Therapien anzuleiten und bleibt eine wichtige Messeinheit für infektiöse Erkrankungen. In jüngerer Zeit wurde die Mutanten-Präventions-Konzentration (MPC) als neue Maßeinheit für die *in vitro* Empfindlichkeit oder Resistenz beschrieben, sie basiert auf Untersuchungen von größeren bakteriellen Inocula, i.e. $>10^9$ cfu/ml – so wie jene, die mit so manchen Infektionen beim Menschen und bei Tieren assoziiert werden. Die MPC definiert die niedrigste Wirkstoffdosis, die notwendig ist, um das Wachstum der am wenigsten empfindlichen Zelle, die in Bakterienpopulationen mit hoher Dichte vorkommt, zu hemmen. MPC Untersuchungen können bei Mikroorganismen durchgeführt werden, die als sensitiv auf einen Wirkstoff gelten, welcher mittels MIC ausgetestet wurde. Das Mutanten-Selektionsfenster (MSW) definiert die „Gefahrenzone“ für therapeutische Wirkstoffkonzentrationen. Eine Verminderung der Zeit, während der die Wirkstoffkonzentration im MSW bleibt, könnte dazu beitragen, die Wahrscheinlichkeit einer Selektion auf Resistenz während der Behandlung zu verringern. Das MSW wird eingegrenzt durch die MIC und MPC Werte und definiert durch die Breite der Wirkstoffkonzentration zwischen den gemessenen MIC und MPC Werten. Die MPC Werte könnten unter Bedacht auf die Pharmakologie des Wirkstoffs, eine Vorhersage der Wahrscheinlichkeit einer Resistenzselektion, wenn Bakterien während der Behandlung von infektiösen Krankheiten antimikrobiellen Wirkstoffen ausgesetzt sind, erlauben. In unserer heutigen Umwelt, sollte eine Resistenzprävention ein Ziel der antimikrobiellen Behandlung darstellen.